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SHORT COMMUNICATION

Is gastrointestinal plasticity in king quail (*Coturnix chinensis*) elicited by diet-fibre or diet-energy dilution?

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ABSTRACT

Phenotypic plasticity of organ size allows some animals to manage fluctuations of resource quality or availability. Here, we examined the phenotypic plasticity of the gastrointestinal tract of king quail (*Coturnix chinensis*) in a diet-fibre manipulation study. Quail were offered either a control low-fibre (high-quality) food (8.5% neutral-detergent fibre; NDF), or one of two experimental diets of higher fibre contents of 16% NDF (i.e. low-quality food). To examine whether phenotypic plasticity of organ size was associated with the fibre content per se, or as a consequence of diluting the diet energy contents by adding fibre, one of the high-fibre feeds was 'balanced' with additional energy to match that of the low-fibre control diet. Total empty dry mass of the gastrointestinal tract was significantly heavier among birds offered the unbalanced high-fibre diet as compared with those offered the control diet, with birds offered the fibrous but energy-balanced diet having guts of intermediate size. The heavier entire-gut mass (dry) of quail offered the unbalanced high-fibre diet was associated mainly with these birds having significantly heavier gizzards. Notably, the larger gizzard in the birds offered the unbalanced high-fibre diet was associated with marked increases in their metabolisability (digestion) of diet fibre. Our findings suggest that the available energy in the diet may be more important for eliciting phenotypic changes in the gut of these herbivorous birds rather than simple physical effects of diet fibre on feed intakes or on muscular compensation to fibrous ingesta.

KEY WORDS: Quail, *Coturnix chinensis*, Phenotypic plasticity, Dietary fibre, Energy dilution, Digestive physiology, Gastrointestinal tract, Gastroliths, Gizzard

INTRODUCTION

Phenotypic plasticity of the avian gastrointestinal tract (gut) has been demonstrated for numerous species. For many avian herbivores the gut is especially responsive to changes in diet quality, but the physical and biochemical mechanisms that drive this plasticity are uncertain (Piersma and Lindström, 1997; Starck, 2005). Diet quality is important for vertebrate herbivores because they lack the ability to breakdown the hard-to-digest, fibrous components of vegetation auto-ezymatically (Barboza et al., 2009). Consequently, avian herbivores have been shown to increase the size of some intestinal organs, particularly the gizzard and paired caeca, to assist mechanical breakdown and the microbial-assisted fermentation of plant fibre that typically contains high proportions of cellulose, hemicellulose and lignin (Barboza et al., 2009). As such, a common method for investigating gut plasticity in herbivorous birds involves manipulating diet fibre levels by diluting high-quality, low-fibre feeds with increasing levels of hard-to-digest, fibrous material. In this regard, diet dilution, and specifically diet-energy dilution, refers to the decrease in easily accessible nutrients (e.g. soluble cell contents) that accompanies any increase in the contents of hard-to-digest, fibrous material [i.e. digestible rather than gross energy contents (see Barboza et al., 2009)]. However, to the best of our knowledge, no previous studies have been able to distinguish potential effects of diet-energy dilution from any effects associated with changes in food intake rates or as a consequence of any physical attributes that fibre might have on gut muscle. Therefore, using three novel diet formulations, we isolated the effects of diet-fibre contents and energy dilution on the food intakes, metabolisability and gastrointestinal plasticity of a small herbivorous bird, the king quail (*Coturnix chinensis* Linnaeus 1766).

The three diets offered to our quail (Table 1) were either a high-quality, low-fibre (LF) food containing approximately 8.5% neutral-detergent fibre (NDF; mainly cellulose, hemicellulose and lignin) and approximately 3% acid-detergent fibre (ADF; mainly cellulose and lignin), or one of two high-fibre (low-quality) diets, each containing approximately 16% NDF and 6–7% ADF. To examine whether changes in organ size were associated with the fibre content of the diets per se, one of the high-fibre diets was balanced with additional energy (HFB) to match the energy contents of the LF control diet, but the second high-fibre diet remained unbalanced (HFU), and was therefore energy-dilute. Diets were the same in all other respects (Table 1), and were based on a standard poultry formulation (see Materials and methods).

RESULTS AND DISCUSSION

The first key finding of our study was that morphological adjustments of the quail gut could be driven by energy-dilution effects, independent of food intake and not solely as a consequence of diet fibre. Specifically, quail offered the energy-dilute HFU diet had heavier guts (entire dry mass) than those offered the higher quality LF diet, but not the HFB diet (Table 2). These differences were driven mainly by the significantly heavier gizzard of the HFU-fed birds (wet and dry masses), being 1.4 times that of the LF-fed birds, and 1.2 times heavier than that of the HFB-fed birds, though the latter group's gizzards were not significantly different from either the LF- or HFU-fed birds. These results are suggestive of a graded response in organ plasticity, whereby the need for a larger gizzard by the HFB-fed birds was apparently tempered by access to more easily accessible energy content of their diet.

Importantly, the larger gizzards of the HFU-fed birds apparently allowed them to maintain body mass and body condition (fat mass) throughout the experiment (Table 3). By the end of the experiment, there were no significant differences in the abdominal fat masses between the LF- or HF-fed quails (i.e. HFB or HFU; Table 2). Likewise, the HFU-fed quail maintained feed intakes (dry and organic matter) comparable to those of LF- and HFB-fed quail (Table 1), in support of Starck's (Starck, 1999) suggestion that

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Received 13 January 2014; Accepted 19 February 2014

The Journal of Experimental Biology (2014) doi:10.1242/jeb.102418

Table 1. Formulations and contents for the low-fibre (LF), high-fibre balanced (HFB) and high-fibre unbalanced (nutrient diluted; HFU) diets offered to king quail

	LF	HFB	HFU
Contents as fed (%)			
Wheat – feed	72.5	44.6	54.4
Soybean meal	15.2	13.2	11.4
Wheat – bran	–	25	25
Corn oil	2.1	7.1	1.6
Salt	0.14	0.17	0.11
Sodium bicarbonate	0.21	0.17	0.16
DL methionine	0.08	0.08	0.06
Lysine HCl	0.02	–	0.02
Limestone	8.8	8.8	6.6
Dicalcium phosphate	0.77	0.77	0.58
Vitamin premix	0.2	0.2	0.2
Composition (mean ± s.d.)			
Dry matter (DM; %)	92.1±0.2	92.0±0.3	92.3±0.3
Organic matter (OM; %)	85.5±0.2	81.7±0.3	85.2±0.2
Gross energy (kJ g^{-1} OM)	16.4±0.2	18.5±0.8	17.2±0.4
Metabolisable energy (kJ g^{-1} OM)*	11.5±0.6	10.8±1.0	11.5±0.7
Nitrogen (% OM)	3.07±0.10	2.96±0.09	3.01±0.10
Neutral detergent fibre (% OM)	8.5±0.5	15.6±0.5	16.7±2.5
Acid detergent fibre (% OM)	3.0±0.0	5.9±0.2	6.9±0.1

*Estimated *post hoc* based on data presented in Table 1.

vertebrate gut plasticity may be largely independent of food intake rates. Additionally, we provide the first experimental evidence that diet energy composition (or energy dilution) may be crucially important for eliciting phenotypic plasticity of the vertebrate gut rather than the fibre content per se.

The second key finding of our study was that the HFU-fed quail had markedly higher metabolisability of plant fibres (NDF and ADF) compared with quail offered the LF or nutrient-balanced HF diets (Table 3). Although the apparent metabolisability of organic matter by the LF-fed quail was on average higher than that of the HFB and HFU-fed quail, these differences were relatively minor compared with strikingly high levels of fibre digestion by the HFU-fed quail. Overall, the HFU-fed birds apparently metabolised 42% of ingested NDF and 21% of ingested ADF, levels that were approximately twice those for the LF- and HFB-fed birds (Table 3).

The main sites for microbial-assisted fermentation in herbivorous birds are the paired caeca, and marked increases in caecal mass have been observed in numerous bird species when feeding on high-fibre diets (e.g. Moss, 1974). However, the mass of the generally heavier paired caeca of our HFU-fed quail was not statistically significantly different from that of the LF- or HFB-fed quail, although these data were quite variable (Table 2). Moreover, it is entirely possible that

Table 2. Mean (±s.d.) organ, abdominal fat and liver masses from king quail offered low-fibre (LF; *n*=6), high-fibre balanced (HFB; *n*=6) and high-fibre unbalanced (HFU; *n*=6) diets

	LF	HFB	HFU	Diet *F* or *H**	Diet *P*
Entire gut					
Wet (g)	2.54±0.61	2.86±0.41	3.16±0.43	2.36	0.13
Dry (mg)*	691.5 ± 59.0^{a}	$781.5\pm17.1^{a,b}$	912.8 ± 43.4^{b}	9.06	0.01
Crop					
Wet (mg)	68.7±15.4	85.5±28.4	103.3±22.1	3.52	0.06
Dry (mg)	16.7±5.5	20.3±7.5	23.8±7.7	1.60	0.24
Proventriculus					
Wet (mg)	179.0±23.9	202.1±32.0	228.3±50.1	2.66	0.10
Dry (mg)	43.7±6.6	49.3±7.8	56.8±12.2	3.10	0.08
Gizzard					
Wet (g)	1.16 ± 0.11^{x}	$1.39\pm0.28^{x,y}$	1.64 ± 0.21^{y}	7.79	0.005
Dry (mg)	340.2 ± 27.0^{a}	$404.2\pm93.5^{a,b}$	473.3 ± 48.2^{b}	6.76	0.008
Small Intestine					
Wet (mg)	808.0±201.9	896.9±231.8	1113.3±244.1	2.88	0.09
Dry (mg)	235.0±34.2	247.0±67.0	289.8±62.0	1.57	0.24
Caeca					
Wet (mg)*	137.8±9.9	143.6±51.3	171.3±36.5	4.99	0.08
Dry (mg)	39.0±5.5	41.5±17.0	48.3±13.4	0.84	0.45
Rectum-cloaca					
Wet (mg)	77.7±14.3	78.8±23.0	88.4±14.7	0.65	0.54
Dry (mg)	17.0±4.9	19.2±6.1	20.7±4.2	0.77	0.48
Liver (wet; g)	$1.19\pm0.16^{a,b}$	1.30 ± 0.15^{b}	1.50 ± 0.20^{a}	4.8	0.02
Abdominal fat (wet; g)	1.07±0.68	1.04±0.23	0.73±0.23	1.0	0.39

Within a row, means bearing different superscripts are significantly different ($^{a,b}P<0.05$; $^{x,y}P<0.001$).
*Kruskal–Wallis *H*-statistic (see Materials and methods).

The Journal of Experimental Biology (2014) doi:10.1242/jeb.102418

Table 3. Mean (±s.d.) intakes and metabolisability by king quail offered low-fibre (LF; *n*=6), high-fibre balanced (HFB; *n*=6) and high-fibre unbalanced (HFU; *n*=6) diets

	LF	HFB	HFU	Diet *F*	Diet *P*
Body mass					
Initial (g)	52.8±4.8	50.9±2.7	50.8±3.8	0.5	0.62
Change (% initial)	–0.9±5.2	5.4±6.8	2.8±5.0	1.8	0.19
Dry matter					
Gross intake (g day^{-1})	6.56±0.38	6.55±0.61	6.82±1.03	0.27	0.76
Metabolisability (%)	72.9±4.8^{a}	61.4±4.7^{b}	68.7±2.9^{a}	11.2	0.001
Organic matter*					
Gross intake (g day^{-1})	5.91±0.35	5.75±0.54	6.14±0.92	0.55	0.59
Metabolisability (%)	76.6±3.9^{a}	66.9±4.7^{b}	70.68±2.2^{b}	10.1	0.002
Energy					
Gross intake (kJ day^{-1})	107.3±6.3	121.2±11.3	117.2±17.6	1.95	0.18
Metabolisability (%)	74.7±4.3^{a}	68.4±4.0^{b}	71.8±2.5^{b}	4.28	0.034
Nitrogen					
Gross intake (mg day^{-1})	201.3±11.8	194.2±18.1	205.4±30.9	0.41	0.67
Metabolisability (%)	37.3±10.1	28.0±12.0	29.0±5.3	1.71	0.21
Neutral detergent fibre					
Gross intake (mg day^{-1})	557±32^{a}	902±84^{b}	1088±164^{c}	59.9	$<1\times10^{-4}$
Metabolisability (%)	24.4±17.4^{a}	19.0±11.0^{a}	48.4±11.4^{b}	7.99	0.004
Acid detergent fibre					
Gross intake (mg day^{-1})	174±10^{x}	304±14^{y}	345±52^{y}	66.8	$<1\times10^{-4}$
Metabolisability (%)	14.9±21.7^{a}	9.8±12.7^{a}	41.8±13.0^{b}	6.65	0.008

Within a row, means bearing different superscripts are significantly different ($^{a,b,c}P\leq0.05$; $^{x,y}P<1\times10^{-4}$).
*Organic matter=dry mass – ash (see Materials and methods).

the differences in caecal masses for the HFU-fed birds were biologically relevant, particularly when other intestinal features are considered. For example, the HFU-fed birds tended also to have heavier proventriculus tissue (wet and dry masses; Table 2). The avian proventriculus is proximal to the gizzard (or ventriculus), and is the main acid-secreting organ, but there is evidence that increased acid digestion, along with greater mechanical action in the gizzard, improves fibre degradation (Svihus, 2011). Moreover, mechanical action of the avian gizzard is boosted by gastroliths (or gizzard rocks/stones), and these tended to be more numerous (P=0.06) and had a heavier overall mass (0.14 g) in the HFU-fed birds (supplementary material Fig. S1). It is also possible that changes to the caecal microbial community composition or population sizes could have affected higher fibre metabolisability by the HFU-fed birds. Nonetheless, it is apparent that the high metabolisability of fibre by the HFU-fed birds aided their maintenance of body condition despite the challenging diet. As such, we present tangible evidence for improved fibre digestion in an avian herbivore associated with morphological plasticity of the gut.

Presumably, the larger gizzard of the HFU-fed quail facilitated mechanical and fermentative digestion in our quail by improving fibre particle-size reduction, with the aid of gastroliths. In this regard, food bulkiness may present an important mechanism activating phenotypic changes of the vertebrate gut. Other studies have demonstrated that increases of structural complexity of diets (i.e. increases in hard-to-digest fibre) increase the volume of gizzard digesta, in addition to increases of gizzard tissue mass (Svihus, 2011). Larger particles are generally retained in the avian gizzard until they are reduced below a threshold particle size. For example, in domestic chickens, particles typically pass from the gizzard only once they are reduced to 0.5−1.5 mm (Moore, 1999). Such a threshold particle size for passage from the king quail gizzard is uncertain, but it is worth noting that our HFU-fed birds' gizzards contained 1.3 times the wet contents of the LF-fed birds, the values being 5.7±0.1 and 4.3±0.1 g for HFU- and LF-fed birds, respectively (Tukey's HSD, P<0.05). Furthermore, the HFB-fed birds' gizzard masses (wet and dry; Table 2) and wet contents (4.6±0.2 g) were intermediate between those of the LF- and HFU-fed birds, suggesting that food bulk or particle size had some effect on gizzard plasticity. Nonetheless, our central conclusion is that, in addition to any textural-, particle-size- or fibre-bulk-associated effects, phenotypic plasticity of the avian gut can be elicited by the energy composition of the diet offered, or that of the subsequent digesta and absorbta.

MATERIALS AND METHODS

Ethics

All experimental procedures were approved by the University of Wollongong's Animal Ethics Committee (protocol no. AE11/15), in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Housing and animal management

Female king quail (N=18 sexually mature, 2–3 year olds; *Coturnix chinensis*) were obtained from a commercial supplier (Andrew's Quail and Pet Palace, Smithfield, New South Wales, Australia). All quail were held at the Ecological Research Centre at the University of Wollongong. Quails were housed individually in mesh-floored plastic cages (30×30×30 cm) and excreta were collected under each cage using a tray lined with non-stick baking paper. Animals were housed in a temperature-controlled facility (22–24°C) at 50–60% relative humidity under a 14 h:10 h light:dark photoperiod (lights on at 06:00 h; full-spectrum UV fluorescent bulbs). All quail were acclimated to housing and regular husbandry procedures (e.g. handling and weighing, daily feed checks and changes, excreta collection) for 3 weeks prior to experimentation. Quail were weighed (±0.1 g) every 3 days throughout acclimation and experimental periods.

Feeding trials

All diets were prepared by The Poultry Research Foundation, The University of Sydney, Australia (Table 1). A standard low-fibre (LF) poultry feed containing 8.5% NDF (mainly hemicelluloses, cellulose and lignin) and 3% ADF (mainly cellulose and lignin) provided all animals with a consistent acclimation diet, and presented a control diet through the experimental period. Two additional diets were used during the experimental period, each

containing higher fibre contents of 16–17% NDF and 6–7% ADF (Table 1). One of the high fibre diets was 'balanced' (i.e. high-fibre balanced; HFB) with corn oil to match the metabolisable energy contents of the LF diet (Table 1). The second high fibre diet was not energy balanced and was therefore energy diluted, or 'unbalanced' (i.e. high-fibre unbalanced; HFU). Aside from differences in total fibre (NDF and ADF), diets were comparable in all other respects, particularly dry matter (DM), organic matter (OM) and nitrogen contents (Table 1).

Following acclimation, animals were randomly assigned to one of the three diets: LF (control), HFB or HFU. For those offered HFB or HFU, transition to the treatment diet occurred incrementally by diluting the LF diet with 50, 70 and 100% of the treatment diet over 3 days, respectively. Once fully transitioned, quail remained on their respective diets for 14 days (N=18 quail; n=6 per treatment), during which daily feed intake was measured (to ±0.01 g). Excreta were collected every 3 days on pre-weighed sections of non-stick baking paper (Castaway easy-bake; Packaging Direct, Wollongong). Samples of feed offered and complete excreta were frozen and stored at −20°C.

Sample analyses

Samples of feed offered and excreta were thawed and thoroughly mixed, and subsamples (ca. 1–2 g) from each quail were bulked individually for the last 9 days of the feeding trial. Bulked excreta and feed subsamples (ca. 1–2 g) were then oven-dried (forced convection) at 55°C until constant mass. Further subsamples (~25% by weight) were dried at 103°C until they reached constant mass to determine complete DM. Dry feed and excreta were ground using a Wiley Mill (0.5 mm screen; Thomas Scientific, Wiley Mini Mill 3383-L40, Swedesboro, NJ, USA). Subsamples (ca. 0.5 g) of ground DM were ashed at 600°C for 5 h in a muffle furnace (Model LCF15-12, LABEC Laboratory Equipment Pty Ltd, Marrickville, NSW) to determine OM (i.e. DM ash).

Fibre contents of feed and excreta were determined using an ANKOM Fibre Analyser (Model A220, ANKOM Technology Corp., Macedon, NY, USA). Subsamples (ca. 0.5 g) of feed and excreta dried at 55°C were analysed in duplicate for NDF and ADF content using the sequential filter-bag technique. Prior to neutral-detergent digestion, samples were treated with 1 ml of heat-stable amylase (Sigma A – 3306; Sigma Aldrich, Sydney) for 80 min to remove starch, and sodium sulphite and decalin were omitted from the neutral-detergent procedure (Van Soest et al., 1991).

Subsamples of ground, dried (at 103°C) feed and excreta were analysed for gross energy content by combusting duplicate subsamples (0.5 g) in an automatic adiabatic bomb calorimeter (Gallenkamp, CBA-305, Gallenkamp and Co. Ltd, UK; calibrated every 15 samples using a benzoic acid standard), and total nitrogen content by combusting duplicate subsamples (200±10 mg) using a Leco CNS-2000 combustion analyser (Leco Inc., St Joseph, MI, USA).

Food intake and apparent metabolisability

Apparent metabolisability (%) of diet components (e.g. DM, energy) was calculated as [(Intake – Excreta) / Intake] × 100, where intake and excreta are in g day^{-1} and contents are per unit of DM or OM (Barboza et al., 2009).

Organ morphology

At the end of each feed trial period, quail were euthanized by CO_2 asphyxiation followed by cervical dislocation and macroscopic dissections were performed immediately. The gastrointestinal tract was removed and cleared of mesentery and fat. Organs (liver, crop, proventriculus, gizzard, small intestine, right and left caeca, and rectum-cloaca) were separated from the entire gut and weighed (±0.001 g) prior to being emptied of contents, rinsed with physiological (0.9%) saline, and re-weighed to determine empty wet mass. Organ lengths were measured using electronic calipers (precision 0.01 mm). Gizzard contents were collected and stored frozen at –20°C for later analysis (contents of the gizzard for one animal from the HFU group was inadvertently discarded). Organs (excluding liver) were dried (forced convection) to constant mass at 95°C.

Statistics

Values presented are means ± s.d. We used an ANOVA to compare organ mass, body mass, gastrolith number and mass, diet component intake and metabolisability across diets. Assumptions for ANOVA were tested using the Ryan–Joiner test for normality and Bartlett's test for homogeneity of variance. To meet the assumptions for ANOVA, some data were log-transformed (ADF intake, gizzard dry mass), and all proportional data were arcsine transformed. Some data sets could not be transformed to meet ANOVA assumptions (caecal wet mass and entire-gut dry mass) and non-parametric Kruskal–Wallis tests were in these cases. Significant differences detected by ANOVA or Kruskal–Wallis (P≤0.05) were further explored using a Tukey's honest significant difference (HSD) *post hoc* test. We used z-tests to determine whether there were significant changes in quail body mass (as a proportion of initial mass compared with a hypothetical change of zero). All analyses were performed using Minitab for Windows (version 15.1.30.0; Minitab Australia).

Acknowledgements

Thanks to Aaron Cowieson and Joy Gill (Poultry Research Foundation, University of Sydney) for diet formulations, and Professor Mike Thompson (School of Biological Sciences, University of Sydney) and William Foley (Research School of Biology, Australian National University) for access to facilities and assistance with analysis of samples. Sincere thanks to Professor Tobias Wang and an anonymous reviewer for their insightful and helpful comments and reviews; it is much appreciated.

Competing interests

The authors declare no competing financial interests.

Author contributions

A.J.M., S.A.W. and S.K.C.J. devised the experiment, S.A.W. and S.K.C.J. performed the experiment, S.A.W. performed sample preparation and analysis, S.A.W. and A.J.M. analysed the data, and A.J.M. and S.A.W. wrote the manuscript.

Funding

This work was carried out without specific funding, but was made possible by the generous support of the University of Wollongong and the University of Wollongong's Institute for Conservation Biology and Environmental Management.

Supplementary material

Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.102418/-/DC1

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